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Formation of Polyelectrolyte Complexes Containing Polysaccharides

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The formation of polyelectrolyte complexes was investigated by using acidic polysaccharides, such as heparin, sulfated methyl cellulose, and carboxymethyl cellulose as a polyanion component, and polyethyleneimine as the polycation component. From conductometric and potentiometric titrations, and turbidity measurement it was pointed out that chemical structures of backbone chains played an important role in morphology of polyelectrolyte complexes and stoichiometry of their reaction.

INTRODUCTION

In our preceding paper,¹⁾ we pointed out that complex formation between carboxymethyl cellulose (CMC) and polyethyleneimine (PEI) was not stoichiometric with respect to the equivalence of ionized groups in each polymer. In this paper, we shall use heparin and sulfated methyl cellulose as the polysaccharide component in polysaccharide-PEI systems.

Glucosidic residues in cellulose derivatives are linked by β -1,4-linkages. The unperturbed chain dimension of cellulose was reported as $7.90P^{1/2}$ (Å),²⁾ in which P is the degree of polymerization. On the other hand, that of amylose was reported as $4.26P^{1/2}$ (Å).³⁾ Glucosidic residues in amylose, heparin, and dextran are linked by α -1,4-linkages. A low-angle X-ray scattering study suggested that heparin behaves as a random coil chain in water.⁴⁾ Polysaccharides linked by β -1,4-linkages are regarded to be less flexible and their chain dimensions in θ solvent are more extended than those of polysaccharides linked by α -1,4-linkages.

Heparin is a naturally occurring acidic polysaccharide. Since its discovery in 1916, a large number of researches have been carried out. Heparin forms complexes with divalent metals, dyes, and proteins, for which heparin behaves as polyanion having high charge density. In particular, complexes of heparin with basic dyes exhibited a large Cotton effect as characteristic of helical conformation.⁵⁾ Desulfation of sulfoamino groups in heparin molecules is easier than O-desulfation. Biological activity of heparin is rapidly reduced by the desulfation of sulfoamino groups.⁶⁾ Jensen⁷⁾ also has suggested that amino nitrogen of heparin plays a fundamental role for anti-clotting action. Therefore, we may presume that carboxyl and sulfoamino groups in heparin play important roles in its reaction with proteins, *e.g.*, anti-clotting action. In heparin there are a considerable number of OH groups besides ionic groups, and they make heparin molecules highly hydrophilic.

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Sulfated methyl cellulose (SMC) used in this work, a sulfated product of methyl cellulose, contains sulfuric, methoxyl, and hydroxyl groups in the chains, though the amount of OH groups is few; thus the affinity of SMC to water is lower than heparin.

The chemical structures of these polysaccharides are shown in Fig. 1, together with that of CMC for comparison. The aim of this work is to elucidate the effect of molecular structure of polyanions on the formation of polyelectrolyte complexes.

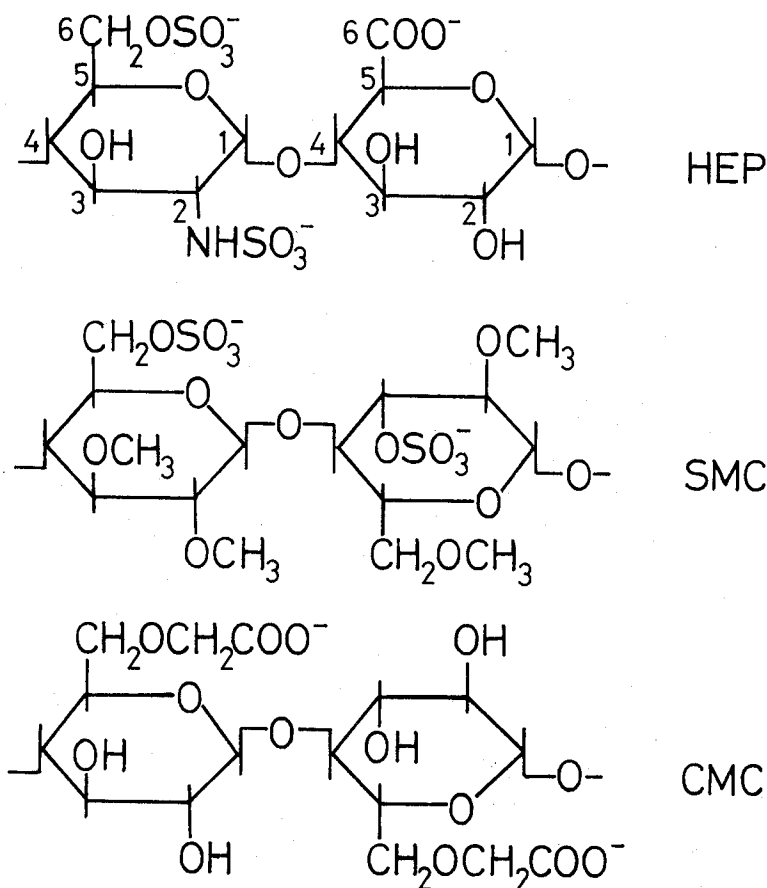


Fig. 1. Chemical structure of HEP, SMC, and CMC.

EXPERIMENTAL

SMC was prepared from methyl cellulose. The methyl cellulose used was supplied from Matsumoto Yushi Seiyaku Inc., and the degree of methylation of which was *ca.* 1.8. Further, it may be regarded that the methyl groups distribute uniformly along the molecular chains.⁸⁾

The sulfation of methyl cellulose was carried out in accordance with the method for starch proposed by Tamba.⁹⁾ Ten grams of methyl cellulose was dissolved in 250 ml of pyridine. The amount of chlorosulfonic acid, added to that solution together with 150 ml of chloroform, was twice the number of hydroxyl groups in

methyl cellulose. The reaction mixture was kept at 50°C for 30 min. After addition of an aqueous solution of NaOH to the mixture, the resultant (SMC-Na) was precipitated by adding acetone, and then washed with methanol. From the potentiometric titration curve of the SMC-Na it was confirmed that the sample behaved as a strong polyanion. SMC was hydrolyzed with HCl and HNO₃, and a BaCl₂ solution was added to the hydrozate. The degree of substitution of SMC was determined as 0.855 from the weight of precipitate BaSO₄.

Heparin sodium was of guaranteed reagent grade, purchased from Nakarai Chemicals Co., Lot No. M3E7945, and assayed at 157 I. U./mg.

PEI and CMC used were the same ones as written in our previous paper.¹³ The degree of substitution of CMC was 0.75. Both samples were deionized by passing through a mixed bed of ion-exchange resins. Conductance water was used throughout the experiment.

The pH measurements for solutions of polyelectrolytes and polyelectrolyte complexes were performed at 25±0.01°C with a Yanagimoto pH meter Type 7.

In the calculations of the degree of dissociation α , activities of H⁺ and OH⁻ ions in a polyelectrolyte solution were assumed to be equal to those in solutions where polyelectrolyte was absent. Concentrations (equil/l) of weak polyelectrolyte in solutions were measured with a Yanagimoto Conductivity Outfit Model MY-8.

The turbidities of solutions, measured with a Shimadzu-Kotaki Photoelectric Nephelotitrator Type NT 3H at the wave length of 436 m μ , were obtained as relative turbidities referred to the turbidity for 6×10⁻³% (w/v) solution of an equivalent mixture of CMC and PEI.

Microscopic photographs of the colloidal aggregate formed from polyelectrolyte complexes were taken with a microscope manufactured by Nihon Kogaku Co.

RESULTS AND DISCUSSION

Heparin (HEP) is regarded as an alternating copolymer composed of D-glucosamine and D-glucuronic acid, in which the sulfuric groups are said to attach to C(6) and C(2) of the former and in a small amount to C(2) of the latter monomers. The content of carboxyl groups, 1.80×10⁻³ equil/g, in heparin determined by conductometric titration agreed nearly with the theoretical value, 1.817×10⁻³ equil/g, calculated by neglecting the presence of sulfuric groups linked to the C(2). Therefore, a sulfuric group at the C(2) is deleted in Fig. 1.

In Fig. 2, the titration curves for HEP and PEI are shown. Curve 1 indicates that the dissociation of carboxyl groups in HEP is rather suppressed in comparison with those¹³ of CMC. Such suppression may be attributable to the presence of many sulfuric ions in HEP. Curve 2 shows that the dissociation of carboxyl groups is accelerated by the addition of microsalt, NaCl, because the repulsive force between ions on the polymer chains is reduced by the presence of the microsalt.

Formation of polyelectrolyte complex between SMC and PEI was highly sensible to pH, polymer concentration, and mixing ratio of polyanion and polycation. In Fig. 3, the pH dependence of turbidity of SMC-PEI complex system is compared

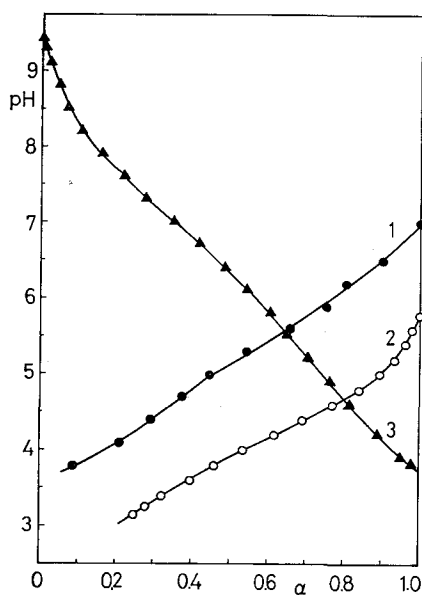


Fig. 2. Titration curves for carboxyl groups of HEP and PEI. Curve 1, 0.0021 *N* HEP in aqueous solution; curve 2, 0.0022 *N* HEP in 0.1 *M* NaCl; curve 3, 0.01 *N* PEI in aqueous solution.

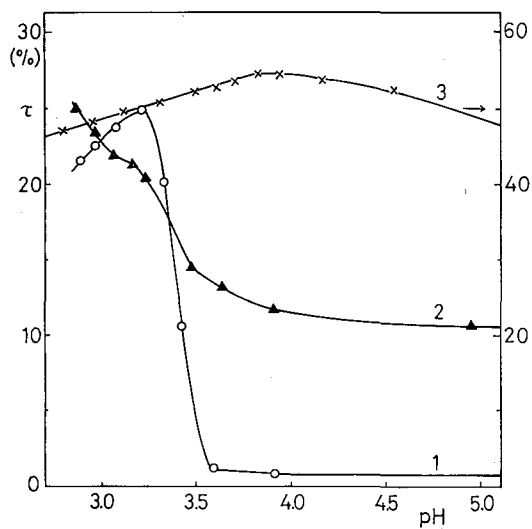


Fig. 3. Effect of pH on the formation of polyelectrolyte complexes. Curve 1, 0.106% SMC-PEI ($R_{PEI}=0.436$); curve 2, 0.00515% HEP-PEI ($R_{PEI}=0.4$); curve 3, 0.00377% CMC-PEI ($R_{PEI}=0.5$).

with those of HEP-PEI and CMC-PEI complex systems. The mixing ratio R_{PEI} is defined as

$$R_{PEI} = C_{PEI} / (C_{PEI} + C_{PHT})$$

in which C_{PEI} and C_{PA} denote the concentrations in equil/l of PEI and acidic polysaccharides, respectively. In the systems of SMC-PEI and HEP-PEI, the complex formation was attained at relatively low pH region, presumably owing to the dissociation of PEI. In SMC-PEI system, however, if the pH is too low, the complex formation was suppressed. Hence the following experiments with SMC-PEI and HEP-PEI were performed at pH=3.15–3.20. In the range of pH from 3.15 to 3.20, PEI is fully dissociated while carboxyl groups in HEP seem not to be dissociated (Fig. 2).

If the complex formations between SMC and PEI, and between HEP and PEI proceed stoichiometrically, then complex formations are to be maximum at $R_{PEI}=0.5$ for the former, and $R_{PEI}=0.4$ for the latter. Relations between the turbidity and the concentration of the polyelectrolyte complex are shown in Fig. 4 for three

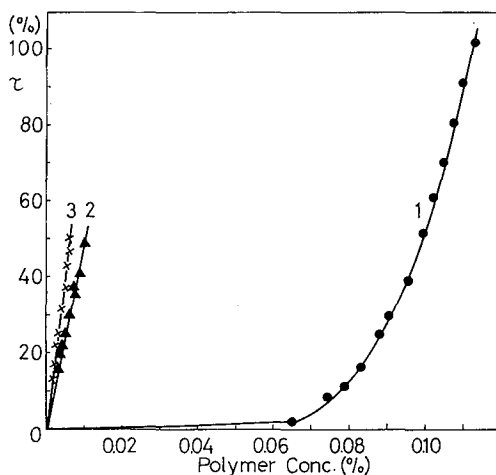


Fig. 4. Relations between turbidity τ and polymer concentration. Curve 1, CMC-PEI ($R_{PEI}=0.5$) at pH=3.15–3.20; curve 2, HEP-PEI ($R_{PEI}=0.4$) at pH=3.1–3.2; curve 3, CMC-PEI ($R_{PEI}=0.5$).

systems, SMC-PEI, HEP-PEI, and CMC-PEI. As is obvious from the figure, the curve for SMC-PEI complex is distinctly different from those for HEP-PEI and CMC-PEI. In the latter two cases, the polymer concentration is in a nearly linear relation against the turbidity, whereas, in the former case, *i.e.*, SMC-PEI, complex formation is not detected by turbidity measurement unless the polymer concentration is sufficiently high. When a concentrated solution of SMC-PEI complex (higher than 0.1%) was allowed to stand overnight, the turbidity of this solution was somewhat decreased and some white sol was precipitated. Such a behavior observed for SMC-PEI system resembles to the liquid-liquid phase separation, *i.e.*, complex

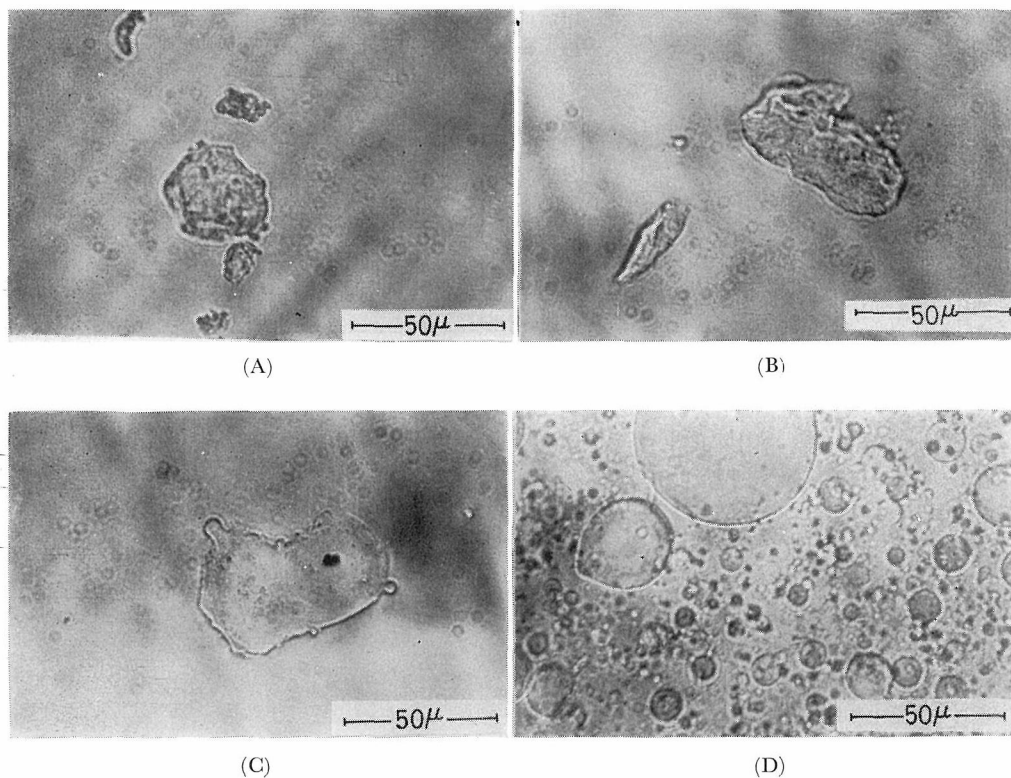


Fig. 5. Micrographs of aggregated particles of polyelectrolyte complexes. A, CMC-PEI ($R_{PEI}=0.5$); B, HEP-PEI ($R_{PEI}=0.4$) at pH 3.15–3.20; C, 0.125% SMC-PEI ($R_{PEI}=0.5$) at pH=3.14; D, droplets formed after standing "C" overnight.

coacervation, as found in the aqueous system of positively and negatively charged polyvinyl alcohol derivatives,¹⁰⁾ rather than the solid-liquid phase separation as found in HEP-PEI and CMC-PEI systems.

In Fig. 5, to confirm such presumption, microscopic photographs of polyelectrolyte complexes are shown. A, B, and C in Fig. 5 are the photographs for CMC-PEI, HEP-PEI, and SMC-PEI complexes, respectively. In A and B, more wrinkles are observed inside the colloidal aggregates than those in C, in which such wrinkles are very few and the shape of droplets are deformed considerably in comparison with droplets formed from a complex of polyvinyl alcohol derivatives.¹⁰⁾ When C is allowed to stand overnight, the shape of aggregated particles becomes spherical as shown in D, which resembles to the coacervate droplets observed in the complex of polyvinyl alcohol derivatives.¹⁰⁾ In the system of SMC-PEI, there was observed a small amount of precipitates as appeared in CMC-PEI system. This kind of precipitates may be regarded as a source of turbidity which appeared at the higher pH region of SMC-PEI system (Fig. 3), at the polymer concentration below 0.06% (Fig. 4) and at the NaCl concentration higher than several mM (Fig. 6).

In Fig. 6, the turbidity is plotted against the ionic strength for systems of HEP-PEI and SMC-PEI. It is pointed out that the turbidity of polyelectrolyte complex

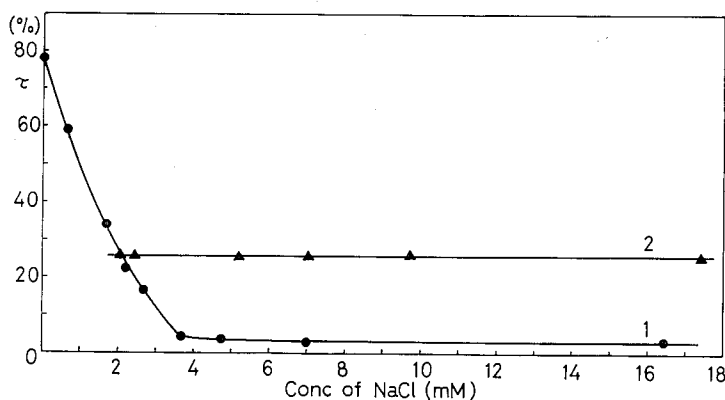


Fig. 6. Dependence of turbidity on ionic strength for complex solutions in pH=3.15–3.20. Curve 1, 0.0961–0.0924% SMC-PEI ($R_{PEI}=0.5$); curve 2, 0.00418% HEP-PEI ($R_{PEI}=0.4$).

formed by the solid-liquid phase separation, such as HEP-PEI, is scarcely affected at low ionic strength region, but that of SMC-PEI is highly affected by the ionic strength, just as coacervate droplets were sensible to the ionic strength. The turbidity of SMC-PEI complex solution decreases at low pH region, as seen in Fig. 3. Such a decrease may be attributed not only to aggregation or coalescence of complex but to the increase of ionic strength due to the addition of HCl.

In Fig. 7, the turbidity of complex solutions is plotted against the mixing ratio R_{PEI} for three systems in which PEI solution is added to acidic polysaccharide solution. Turbidity measurements were carried out after the turbidity reached to the constant value for HEP-PEI system, and 1 min after the mixing for SMC-PEI system.

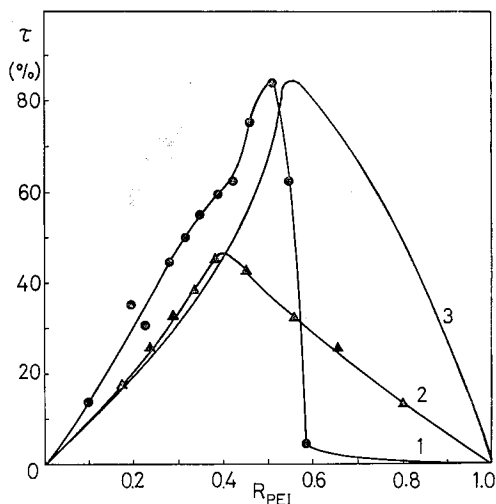


Fig. 7. Turbidity plotted against mixing ratio R_{PEI} . Curve 1, 0.096–0.115% SMC-PEI in pH=3.15–3.20; curve 2, 0.005% HEP-PEI at pH=3.15–3.20; curve 3, obtained from our previous paper.¹⁾

The result on CMC-PEI system is cited from the previous paper.¹⁾ Since $R_{\text{PEI}} = 0.55$ at the maximum turbidity in the CMC-PEI system, the amount of ionizable groups of PEI was larger by 20% than that of CMC. Such a non-stoichiometric nature in complex formation was concluded to be due to that the CMC backbone is not flexible and moreover that the distance between ionized groups in CMC does not correspond to that in PEI geometrically.

On the other hand, curve 2 in Fig. 7 indicates that HEP forms polyelectrolyte complex stoichiometrically with PEI. The mixing ratio R_{PEI} at the maximum turbidity is 0.4, which is in agreement with the theoretical value obtained by assuming stoichiometric relation. HEP is relatively flexible because of α -1,4-linkage, compared with CMC, and stoichiometric complex may be obtained rather easily.

In the case of SMC-PEI system, the amount of complex formed was not linear with the turbidity, and the time dependence of turbidity was large, so that exact discussion may be impossible. However, it may be pointed out from curve 1 of Fig. 7 that the complex formation is largely affected at the mixing ratio R_{PEI} above 0.6. Such a phenomenon as an excess of either polyelectrolyte component suppresses extremely the formation of polyelectrolyte complex has not been known so far. However, taking into account that the complex formation in SMC-PEI is highly sensitive to the ionic strength, the addition of PEI-HCl to SMC at pH=3.15–3.20 may imply a similar effect as the addition of small electrolytes. The authors reported¹¹⁾ that the interaction parameter between polymer and solvent gave a large influence on the complex coacervation. SMC is more hydrophobic than HEP and CMC. Although both of SMC and PEI have high charge densities, they formed complex similar to one prepared from polyvinyl alcohol derivatives. Such a behavior may be attributed to that the backbone chain of SMC is hydrophobic and that the attractive force between both ionic species can not be so strong on account of their chemical structures; SMC has rigid backbone chain owing to β -1,4-linkage and relatively short side chains with ionic groups.

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